CLAIMS

1. A method of detecting a *Plasmodium* malarial agent of humans in a biological sample comprising contacting a probe or primer of the *Plasmodium berghei* extrachromosomal genetic element with said sample or nucleic acid derived therefrom for a time and under conditions sufficient for hybridisation to occur and then detecting said hybridisation using a detection means, wherein the probe or primer is at least 15 nucleotides in length and comprises a nucleotide sequence that is highly conserved in *Plasmodium berghei* and said malarial agent of humans or a complementary nucleotide sequence thereto.

2. The method according to claim 1 wherein the extrachromosomal genetic element is the *Plasmodium berghei* plastid or plastid-like molecule.

3. The method according to claim 2 wherein the probe or primer comprises a nucleotide sequence of the *Plasmodium berghei* plastid-localised LSU rRNA genes or a complementary nucleotide sequence thereto.

4. The method according to claim 3 wherein the probe or primer comprises an LSU rRNA gene or gene fragment that is highly conserved between *Plasmodium berghei* isolate and the malarial agents of humans, wherein said gene or gene fragment comprises a *P. berghei* nucleotide sequence set forth in nucleotides 1147 to 1740 of SEQ ID NO:1 or any one of SEQ ID NOS: 5, 6, 19 or 20 or a complementary sequence thereto.

5. The method according to ~~any one of claims~~ 1 ~~to 4~~ wherein the hybridisation step is performed under low stringency hybridisation conditions.

6. The method according to ~~any one of claims~~ 1 ~~to 4~~ wherein the hybridisation step is performed under moderate stringency hybridisation conditions.

7. The method according to ~~any one of claims~~ 1 ~~to 4~~ wherein the hybridisati n step is

performed under high stringency hybridisation conditions.

8. The method according to ~~any one of~~ claims 1 ~~to 7~~ wherein the detection means used to detect the hybridisation comprises identifying a signal produced by a reporter molecule bound to the probe or primer, wherein the reporter molecule is capable of producing an identifiable signal.

9. The method according to claim 8 wherein the reporter molecule is a radioisotope or a non-isotopic reporter molecule such as biotin.

10. The method according to ~~any one of~~ claims 1 ~~to 7~~ wherein the detection means comprises a polymerase chain reaction (PCR) format using one or more *Plasmodium* genus-specific primers or primer pairs.

11. The method according to claim 10 wherein the PCR format comprises RT-PCR.

12. The method according to claim 10 ~~or 11~~ wherein the genus-specific primer pairs comprise SEQ ID NOs: 5 and 6.

13. The method according to ~~any one of~~ claims 1 ~~to 12~~ wherein the *Plasmodium* malarial agent of humans is selected from the list consisting of *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*.

14. The method according to ~~any one of~~ claims 1 ~~to 13~~ wherein the biological sample comprises blood or a derivative thereof.

15. The method according to claim 14 wherein the biological sample comprises dried blood.

16. A method of detecting a particular species of the *Plasmodium* malarial agent of

humans in a biological sample comprising nucleic acid with a *Plasmodium* species-specific extrachromosomal genetic element probe or primer for a time and under conditions sufficient for hybridisation to occur and then detecting said hybridisation using a detection means, wherein the probe or primer is at least 15 nucleotides in length and comprises a nucleotide sequence that hybridises specifically to the nucleic acid of the extrachromosomal genetic element of one species of a *Plasmodium* malarial agent of humans.

17. The method according to claim 16 wherein the probe or primer hybridises specifically to nucleic acid of the mitochondrial *coxI* gene of *Plasmodium falciparum* or *Plasmodium vivax* or *Plasmodium ovale* or *Plasmodium malariae*.

18. The method according to claim 17 wherein the probe or primer hybridises specifically to a non-conserved or less-conserved nucleotide sequence of the mitochondrial *coxI* gene of *Plasmodium falciparum* or *Plasmodium vivax* or *Plasmodium ovale* or *Plasmodium malariae* as determined from a nucleotide sequence alignment of several isolates of said species.

19. The method according to claim 18 wherein the probe comprises a nucleotide sequence that set forth in SEQ ID NOs: 21 or 22 or a complementary nucleotide sequence thereto.

20. The method according to claim 18 wherein the primer comprises a nucleotide sequence set forth in any one of SEQ ID NOs: 11-18.

21. The method according to ~~any one of~~ claims 16 ~~to 20~~ wherein the hybridisation step is performed under low stringency hybridisation conditions.

22. The method according to ~~any one of~~ claims 16 ~~to 20~~ wherein the hybridisation step is performed under moderate stringency hybridisation conditions.

23. The method according to ~~any one of~~ claims 16 ~~to 20~~ wherein the hybridisation step is performed under high stringency hybridisation conditions.

24. The method according to ~~any one of~~ claims 16 ~~to 20~~ wherein the detection means used to detect the hybridisation comprises identifying a signal produced by a reporter molecule bound to the probe or primer, wherein the reporter molecule is capable of producing an identifiable signal.

25. The method according to claim 24 wherein the reporter molecule is a radioisotope or a non-isotopic reporter molecule such as biotin.

26. The method according to ~~any one of~~ claims 16 ~~to 23~~ wherein the detection means comprises a polymerase chain reaction (PCR) format using one or more *Plasmodium* species-specific primers or primer pairs.

27. The method according to claim 26 wherein the PCR format comprises RT-PCR.

28. The method according to claims 26 ~~or 27~~ wherein the *Plasmodium* species-specific primer pairs are selected from the group consisting of: SEQ ID NOs: 11 and 12; SEQ ID NOs: 13 and 14; SEQ ID NOs: 11 and 15; SEQ ID NOs: 11 and 16; SEQ ID NOs: 11 and 17; and SEQ ID NOs: 16 and 18.

29. A method of detecting *Plasmodium falciparum* or ~~or~~ *Plasmodium vivax* or *Plasmodium ovale* or *Plasmodium malariae* in a sample comprising blood or a derivative of a blood sample such as dried blood, comprising:

(i) detecting *Plasmodium* LSU rRNA gene sequences in said sample by:

(a) hybridising a nucleic acid probe comprising the *P. berghei* nucleotide sequence set forth in SEQ ID NOs: 19 or 20 or a complementary nucleotide sequence to said sample or nucleic acid derived therefrom; or

(b) amplifying LSU rRNA gene sequences from said sample or nucleic acid derived therefrom in a PCR format using the primer pair comprising SEQ ID NOs: 5 and 6; and

(ii) detecting specific *Plasmodium falciparum* or *Plasmodium vivax* or *Plasmodium*

ovale or *Plasmodium malariae* mitochondrial *coxI* gene sequences in said sample by:

(a) hybridising a nucleic acid probe comprising the nucleotide sequence set forth in SEQ ID NOS: 21 or 22 or a complementary nucleotide sequence to said sample or nucleic acid derived therefrom; or

(b) amplifying *coxI* gene sequences from said sample or nucleic acid derived therefrom in a PCR format using one or more primer pairs selected from the group consisting of: SEQ ID NOs: 11 and 12; SEQ ID NOs: 13 and 14; SEQ ID NOs: 11 and 15; SEQ ID NOs: 11 and 16; SEQ ID NOs: 11 and 17; and SEQ ID NOs: 16 and 18.

30. A method of detecting *Plasmodium falciparum* in a sample comprising blood or a derivative of a blood sample such as dried blood, comprising detecting specific *Plasmodium falciparum* mitochondrial *coxI* gene sequences in said sample by amplifying *coxI* gene sequences from said sample or nucleic acid derived therefrom in a PCR format using one or more primer pairs selected from the group consisting of: SEQ ID NOs: 11 and 15; SEQ ID NOs: 11 and 16; SEQ ID NOs: 11 and 17; and SEQ ID NOs: 16 and 18.

31. A method of detecting *Plasmodium vivax* in a sample comprising blood or a derivative of a blood sample such as dried blood, comprising detecting specific *Plasmodium vivax* mitochondrial *coxI* gene sequences in said sample by amplifying *coxI* gene sequences from said sample or nucleic acid derived therefrom in a PCR format using one or more primer pairs selected from the group consisting of: SEQ ID NOs: 11 and 12; and SEQ ID NOs: 13 and 14.

32. An isolated probe or primer of the *Plasmodium berghei* plastid or plastid-like extrachromosomal genetic element for the genus-specific detection of *Plasmodium ssp.* selected from the group consisting of:

(i) a probe or primer comprising a nucleotide sequence set forth in any one of SEQ ID NOs: 1-6, 19 or 20 or a complementary nucleotide sequence thereto, wherein said pr be or primer is at least 15 nucleotides in length; and

(ii) a probe or primer comprising a conserved sequence of at least 15 nucleotides in length obtainable from the alignment of nucleotide sequences set forth in Figure 9 or a complementary nucleotide sequence thereto.

33. The isolated probe or primer according to claim 32 wherein the nucleotide sequence of said probe or primer is determined by the method of:

(i) determining the nucleotide sequence of one or more regions of the *P. berghei* plastid or plastid-like extrachromosomal genetic element;

(ii) aligning the nucleotide sequence of various *Plasmodium* species according to the Needleman and Wunsch algorithm, wherein said other species is a malarial agent of humans; and

(iii) selecting the highly-conserved nucleotide sequences between *P. berghei* and said other species.

34. The isolated probe or primer according to claim 33 wherein aligning the nucleotide sequence of said one or more regions with the nucleotide sequence(s) of the corresponding region(s) of another *Plasmodium* species according to the Needleman and Wunsch algorithm comprises aligning the LSU rRNA gene sequences of *P. berghei, P. ovale, P. falciparum, P. vivax and P. malariae*.

35. An isolated probe or primer of a *Plasmodium coxI* gene for the species-specific detection a *Plasmodium* malarial agent of humans selected from the group consisting of:

(i) a probe or primer comprising the nucleotide sequence set forth in any one of SEQ ID NOs: 11-18 or 21 or 22 or a complementary nucleotide sequence thereto, wherein said probe or primer is at least 15 nucleotides in length; and

(ii) a probe or primer comprising a non-conserved sequence of at least 15 nucleotides in length obtainable from the alignment of nucleotide sequences set forth in Figure 10 or a complementary nucleotide sequence thereto.

36. The isolated probe or primer according to claim 35 wherein the nucleotide sequence

of said probe or primer is determined by the method of:

(i) determining the nucleotide sequence of the *coxI* genes of several isolates of different species of *Plasmodium* malarial agents of humans;

(ii) aligning the nucleotide sequences of said *coxI* genes according to the Needleman and Wunsch algorithm; and

(iii) selecting the non-conserved or less-conserved nucleotide sequences between said *coxI* genes; and

(iv) testing the ability of the selected nucleotide sequences for their ability to specifically detect one species of a *Plasmodium* malarial agents of humans in a polymerase chain reaction or hybridisation assay.

37. The isolated probe or primer according to claim 36 wherein aligning the nucleotide sequences of said *coxI* genes according to the Needleman and Wunsch algorithm comprises aligning the *coxI* gene sequences of *P. ovale, P. falciparum, P. vivax and P. malariae* and wherein the selected non-conserved or less-conserved nucleotide sequence is tested for its ability to distinguish between one of said species and the remainder of said species.

38. An isolated *Plasmodium falciparum* species-specific primer pair selected from the group consisting of: SEQ ID NOs: 11 and 15; SEQ ID NOS: 11 and 16; SEQ ID NOs: 11 and 17; and SEQ ID NOs: 16 and 18.

39. An isolated *Plasmodium vivax* species-specific primer pair selected from the group consisting of: SEQ ID NOs: 11 and 12; and SEQ ID NOs: 13 and 14.

40. A kit for the detection of a *Plasmodium* malarial agent of humans in a biological sample comprising one or more of the isolated probes or primers according to any one of claims 33 to 38 or ~~the primer pairs according to claims 39 or 40~~ and one or more reaction buffers suitable for use in a nucleic acid hybridisation reaction or polymerase chain reaction.

41. The kit according to claim 40 further comprising a *Plasmodium* nucleic acid molecule

positive standard.

42. A kit for the detection of a *Plasmodium* malarial agent of humans in a biological sample comprising one or more of the isolated probes or primers according to ~~any one of~~ claims 34 ~~to 37 or the primer pairs according to claims 38 or 39~~ and a *Plasmodium* nucleic acid molecule positive standard.

43. The kit according to ~~any one of~~ claims 40 ~~to 42~~ comprising one or more isolated primer pairs, wherein both primers of a primer pair is provided in the same compartment at a relative concentration suitable for the amplification of plastid DNA or plastid-like DNA or mitochondrial *coxI* sequences from a *Plasmodium* malarial agent of humans contained in a blood sample or derivative thereof.

44. The kit according to claim 43 wherein the relative concentration of primers is suitable for the amplification of LSU rRNA or *coxI* sequences from a *Plasmodium* malarial agent of humans contained in a dried blood spot.